Analysis of Bio-priming on Growth Parameters and N_2-fixation Capability of Leguminous Crops under Fluoride Stress Conditions

A Synopsis

Submitted in

partial fulfillment for the degree

of

Doctor of Philosophy

(Life Science & Biotechnology)

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2022

INTRODUCTION

The advent of technology allowed researchers to study more about the microbial populations and their beneficial and harmful effects on organisms and plants, and Kloepper and Schroth (1978) the first time used the term plant growth promoting rhizobacteria (PGPR) explaining them as bacteria which are closely related to the rhizosphere.

Agriculture in the 21st century is facing multiple challenges, such as those related to soil fertility, biotic and abiotic stress, climatic fluctuations, environmental degradation, urbanization, and the increase in food demand and security for the increasing world population. For many last decades, Crop production has been facing serious threats due to various chemicals, and biotic and abiotic stresses. Feeding the growing population and enhancing agricultural production on limited land areas are significant challenges for researchers and farmers in the current era.

In addition, present agricultural practices, such as the use of chemical fertilizers, pesticides, herbicides, and unpracticed irrigation with untreated wastewater, pose serious threats to the environment and cause soil degradation and water stress conditions due to polluted underground water bodies [Appl. Geogr. et al 2012]. Moreover, urbanization and industrialization have caused a significant reduction in the agricultural area and yields, which has further motivated scientists to develop sustainable strategies to increase crop yields from the already shrinking cropped area [Niamat, 2019; B. Naveed, M, 2019]. Therefore, adopting alternative and scientific approaches in today's agriculture is necessary for the safety and ensuring environmental sustainability and future food security.

Towards a sustainable agricultural vision for future generations, crops produced need to be equipped with disease resistance, heavy metal stress tolerance, salt tolerance, drought tolerance, and better nutritional value. To fulfill the above-desired crop properties and requirements, one possibility is to use soil microorganisms/microbes (bacteria, fungi, algae, *etc.*) that increase the nutrient uptake capacity and water use efficiency in water stress areas. [Godfray, H.C.J.; Beddington, J.R.; Crut,2010].

Among these potential soil microbes, bacteria known as plant growth-promoting rhizobacteria (PGPR) are the most promising. Seeds treated with plant PGPRs are an effective bio-priming method to introduce beneficial microbial inocula into the soil. Bio-priming is a type of seed treatment that employs biological entities/microbes, which involves the hydration of seeds and inoculation with beneficial microorganisms. Mainly, the seed bio-priming technique improves the seed quality, germination, viability, growth promotion, vigor index, production, and subsequent disease resistance by enhancing the uniform speed of germination and production of other growth regulators which works as immunity inducers.

In this sense, PGPR may be used to enhance plant health and immunity, therefore, promoting the plant growth rate without environmental contamination [Saharan, B.S.; Nehra,2011].

PGPR shows an important role in the sustainable agriculture industry and improves crop yield. The increasing demand for crop production with a significant reduction of various

synthetic chemical fertilizers, pesticides and herbicides use is a big challenge nowadays for farmers. The use of PGPR has been proven to be an environmentally sound way of increasing crop yields by facilitating plant growth through either a direct or indirect mechanism.[Danish, S.; Zafar-Ul-Hye, M.; Mohsin, F.; Hussain,2020]

The mechanisms for plant growth promotion used by rhizobacteria(alias Rhizobium bacteria), which inhabit the rhizosphere (bacteria inside soil), include metabolic adjustments, adjustments in phytohormone levels, root colonization, production of exopolysaccharides, and enhancement of nutrient availability [Ali, M.A,2017; Ismail,2021; Amin,2021; A Review. Cells 2021, 10, 1551.]. These rhizobacteria also indirectly improve plant growth and development by inducing plant resistance to various biotic and abiotic stresses, such as pathogen attack and heavy metal contamination, using such mechanisms as the production of antibiotics, induction of induced systemic resistance, rhizosphere competence, and production of antagonistic substances for biocontrol.[Ital. J. Agron. 2019]

There are numerous studies about the beneficial attributes of PGPR, such as increasing the bio-availability of various soil nutrients for plant growth and development and stimulating phytohormones for example auxins, gibberellins, and cytokinins, abiotic and biotic stress management of plants by producing many metabolites including ACC-deaminase and biocontrol agents such as antibiotics, HCN (Hydrogen cyanide), siderophore and lytic enzymes production, which in turn enhancing the speed and efficiency of seed germination and their percentage also, proliferation of root growth, ability to withstand in contaminated soils, and soil structure (Basu et al., 2021).

Bio-priming methods using PGPR inoculants are becoming more common and relevant in modern agriculture as an alternative to chemical treatments. They are more environmentally sustainable and safer for future agriculture and our generation apart from improving plants and soil health. Recently, it has been employed as an alternative technique for eradicating numerous soil- and seed-borne diseases. The term "bio-priming of seeds" refers to a common strategy for introducing disease resistance through the use of biocontrol agents. Compared to other known approaches, seed priming with beneficial microbes and biocontrol agents is more effective at managing diseases and pests (Prabha et al., 2019).

A more contemporary method of seed priming is seed bio-priming. Seed priming is a pre-sowing procedure that induces a physiological state that promotes more effective seed germination. The vast majority of seed treatments rely on seed imbibition, which permits the seeds to progress through the first reversible stage of germination but prevents radical protrusion through the seed coat. Dehydrating the seeds allows them to be stored until the final sowing while maintaining their tolerance to desiccation (Stanley et al., 2016). Although there are many other seed-priming strategies used in crops, bio-priming has its unique significance. We have attempted to summarise the review of the literature on seed bio-priming here. Seed bio-priming is a helpful alternative to chemical fungicides for practically all types of crops worldwide.

The experts have revealed that biopriming, which provides a sufficient quantity of bacteria in the seeds, can be employed efficiently in the application of bacteria. The issue of our intended inoculants competing with native bacteria can also be resolved by biopriming because the seeds will already contain our preferred bacteria, lowering the risk of desiccation and the negative impacts of any pesticides used in the field. On the other hand, it might also be a different strategy for applying bacteria to small seeded plants that can ingest the bacterial suspension, causing the bacteria to enter the seed. With regard to certain root rot diseases, biopriming provides equivalent or superior control. Due to financial constraints, there is a need to look for better media for applications, which can be lowered with further investigation. Second, this technique can be used to different crops without having been tested, which will provide a clearer picture of the technology's potential.

Review of Literature

Over the past few years, Bio-priming has attracted a lot of attention .Legumes are exposed to a wide range of abiotic stresses like drought, high temperatures, salinity, cold, alkalinity, ultraviolet light, and mineral toxicity and deficiency. The productivity of crop legumes is frequently impacted by these abiotic stresses.Especially in arid and semi-arid locations where the soil salt concentration is naturally high and rainfall is frequently insufficient for leaching, soil salinity is a significant concern for agriculture in many places [Zhao, G.Q., 2007]. The higher concentration of fluoride in soil cause agricultural problem for farmers, and the higher amount in the soil is due to more fluoride concentration in underground water which uses for irrigation purpose. This fluoride accumulated in the soil and cause problem in legume formation in leguminous crops. This review focuses on the techniques utilised for biopriming, as well as the function this technology plays in enhancing agricultural productivity and stress tolerance (Panda and Khan, 2009).

Kasim et al. (2013) Biopriming was utilised by Kasim to examine how it affected the impacts of drought stress. They found that biopriming with the two strains, A. brasilense and B. amyloliquefaciens, enhanced drought tolerance in wheat plants by upregulating genes associated with stress. The role of biopriming has been investigated in a variety of crops utilising various PGPR. Many studies have been done on the role of biopriming in coping with salinity stress, and encouraging findings have been made. The most notable genus utilised in abiotic stress tolerance is Bacillus, which is used in rice, mungbean, chickpea, radish, potato, Kaymak et al. 2009, and (Chakraborty et al.2011).

Mali *et al,* (2014) Research Fluoride can be found in variable amounts in soil, water, air, and even plants. As a result, both plants and animals become toxic. A powerful metabolic inhibitor, fluoride. The test for photosynthetic pigments is therefore the influence of fluoride

concentration (5 ppm, 10 ppm, 25 ppm, 50 ppm, and 100 ppm) (chlorophylls and carotenoids). It was discovered that exposure to fluoride resulted in an increase in total chlorophylls and carotenoids. This demonstrates that through increasing photosynthetic efficiency, the Simarouba glauca plant reduces fluoride stress.

Kaur and Reddy (2014) According to Kaur and Reddy , the phosphate solubilization activity in the phosphate solubilizing bacteria Pantoea cypripedii and P. plecoglossicida was followed by the synthesis of organic acids in the following order: gluconic acid > oxalic acid > succinic acid. In the extracts of pea seeds made from the plants that were treated by the microbial consortium (P. aeruginosa PJHU15 + B. subtilis BHHU100 + T. harzianum TNHU27), high-performance liquid chromatography (HPLC) analysis revealed accumulation of phenolic compounds, including gallic acid, shikimic acid, syringic acid, tannic acid, etc (Jain et al., 2014).

According to Otieno et al. (2015), endophytes, namely Pseudomonas spp., produced gluconic acid that stimulated pea growth in conditions of low P. (nutritional stress). Pseudomonas inoculated plants grew more than uninoculated plants because the acid released in the rhizosphere aided in plant growth.

Agarwal and Chauhan, (2015) The goal of the current study was to examine the effects of various sodium fluoride concentrations on various yields and their bioaccumulation (Hordeum vulgare) in barley. In the pot experiment, plants treated with 18 ppm of NaF showed bioaccumulation of fluoride in plant components, with the highest accumulation occurring in the leaves (9.948 mg/kg) and the lowest accumulation occurring in the grains (6.302 mg/kg). When sodium fluoride was seen in the field in various crop sections, the leaves showed the highest concentration (16.758 mg/kg), while the treated plants and rural water tubes showed the lowest concentration (9.351 mg/kg). The findings demonstrated that the biological accumulation of fluoride in various plant components may be tolerated when groundwater with a high fluoride content is used to irrigate barley plants.

Singh Vivek et al.,(2016) Seed biopriming is a cutting-edge method of seed treatment that involves applying a helpful microbe to the surface of the seed and then hydrating it. A natural alternative to chemical therapy for the many soil- and seed-borne diseases is seed bioprimig. By promoting seed germination and offering protection prior to seedling emergence, seed biopriming improves the first stage of plant development. Trichoderma spp. is a popular

biocontrol agent that inhibits phytopathogens while also promoting plant growth. The effectiveness of biopriming with T. asperellum BHUT8 was assessed in the current study for its ability to promote plant development in pea. The results showed that the treated plants grew more plants than the control plants. In comparison to the control, the shoot length, root length, number of leaves, shot fresh weight, root fresh weight, shoot dry weight, and root dry weight all increased. These increases were 35.29, 96.49, 28.13, 36.10, 146.26, 30.17, and 77.20%, respectively.

S. P. Monalisa and J. K. Beura (2017) At the Department of Seed Science and Technology, OUAT, Bhubaneswar, an experiment was carried out in 2013–14 to improve the seed quality of common bean (Phaseolus vulgaris L.) var. S 9 (local) by using three biocontrol agents: Trichoderma viride, Trichoderma harzianum, and Pseudomonas fluorescence. During 4, 8, 12, and 16 hours of soaking, seeds were bi-oprimed with the biocontrol agents at 40, 50, and 60% concentration. Moreover, seeds were hydro-primed for 4, 8, 12, and 16 hours. Bio priming with P. fluorescent (at 40% concentration and for 4 hours) was closely followed and on par with the best treatment, with improvements in the aforementioned quality metrics of 11.6%, 18.2%, 16.4%, 30.4%, 30.7%, and 56.9%, respectively.

Punam Kumari et al., (2018) Plant growth-promoting rhizobacteria (PGPR) are helpful bacteria that can colonise plant roots and are obtained from the rhizosphere. They are employed to promote plant development or to control disease brought on by various phytopathogens. The fourteen isolates were screened based on their in vitro antagonistic potential activity against the root rot pathogen Rhizoctonia solani, but for the current study, we have chosen two strains of rhizobacteria (Pseudomonas aeruginosa BHU B13-398 and Bacillus subtilis BHU M) through preliminary screening. The unique PGPR features of these isolates, such as the production of phosphate solubilization, ammonia, siderophores, and HCN, were subsequently examined in vitro. Mung bean pot experiments were carried out to assess their capacity to promote plant growth under regulated greenhouse circumstances (14 h of light, 10 h of darkness, and a temperature of 27 2 °C). The findings showed that, in comparison to uninoculated control plants, both isolates (BHU B13-398 and BHU M) improved the growth characteristics of mung bean, including root length, shoot length, root and shoot fresh and dry weight, leaf area, and chlorophyll content. Our findings unequivocally showed that, as compared to the uninoculated control, the isolates BHU B13-

398 and BHU M both showed increases in shoot length and root length of 32.26%, 84.60%, and 13.38%, 61.94%, respectively.

Prabha et al., (2019) Over the past few years, Bio-priming has attracted a lot of attention. Recently, it is used as an alternate method for controlling seed and soil-borne pathogens. Bio-priming of seeds denotes a standard tactic for the introduction of disease resistance and immunity via biological control agents. Priming of seeds with helpful microorganisms and bio-control means has been testified more efficient for the management of diseases and pests as equated to other available methodologies.

Muhammad Sufyan, Muhammad I. Tahir (2020) Among other pulse legumes, the chickpea (Cicer arietinum L.) makes a significant contribution to a nation's economy. Many soil-borne infections have a significant impact on the yield and quality of the produce, however today's use of PGPR as a chemical alternative is quite effective. Using two rhizobacterial isolates, Pseudomonas putida and Pseudomonas fluorescens, the chickpea desi variety (Bittal-98) was primed by hydrobio priming and drum priming techniques. Drum priming (27.16%) and hydro-biopriming (30.5%) both demonstrated efficient disease control and increased growth metrics. The most successful therapy was when both rhizobacteria were applied together i.e. T3 thus reverberating the effect of each other.

Negi Shivangi and Bharat Narender K (2021) In a field experiment conducted in 2017 and 2018, the effect of seed biopriming with various bioagents, such as plant growth-promoting rhizobacteria (PGPR-1), rhizobial biofertilizer (Rhizobium strain B1), and biological control agent (Trichoderma viride), was observed on plant growth, seed yield, and disease incidence in French bean cv. Contender. As compared to carbendazim seed treatments and untreated controls, this treatment combination also dramatically decreased the incidence of important diseases like Rhizoctonia root rot and Angular leaf spot. The results of this inquiry lead to the conclusion that French bean cv. When compared to untreated control and seeds treated with carbendazim at 0.2%, PGPR-1 + Rhizobium strain B1 @ 109cfu/ml for 8 hours was an efficient treatment that dramatically increased plant growth, pod yield, seed yield, seed quality, and seed vigour and decreased disease incidence.

Dr. Dragana Miljakovic (2022) A novel method of seed treatment called bio-priming boosts crop development, yield, and the vitality of the seeds. This study sought to determine if Bradyrhizobium japonicum (commercial strains) and Bacillus megaterium (newly isolated strains) were effective as a single inoculant and co-inoculant during seed bio-priming to enhance seed germination and early seedling growth of two soybean cultivars. The treated seedlings underwent accelerated ageing tests, cold tests, and germination tests (AAT). As comparison to the control, B. megaterium considerably enhanced all metrics in GT and CT, including final germination, shoot length, root length, root dry weight, and seedling vigour index in AAT. Also, as compared to the control, co-inoculation significantly increased all metrics in GT, CT, AAT, and GT except for shoot dry weight, germination energy, shoot length, root length, and seedling vigour index. Also, when compared to unprimed seeds, Br. japonicum significantly enhanced the following characteristics: germination energy, shoot length, shoot dry weight, root dry weight, and seedling vigour index in GT; all parameters in CT; shoot length, root length, and seedling vigour index in AAT. With careful field testing, B. megaterium strains could therefore be employed in soybean bio-priming as a potential single inoculant and co-inoculant.

Dr. Gordana Dušan Tamindžic (2023) Garden peas and other cool-season food legume crops are negatively impacted by heat stress. A common strategy is seed priming, which boosts germination and enhances plant growth and development for improved field performance and higher crop yield. In the present study, we examined the effects of three seed priming treatments—hydropriming (dH2O), osmopriming (2.2% w/v CaCl2), and hormopriming (50 mg L1 salicylic acid—SA)—on germination, initial seedling development, and physiological traits of two novel garden pea cultivars, both under ideal conditions and under heat stress. Garden pea performance was improved by seed priming with water, calcium chloride, and sulphur dioxide (H2O, CaCl2, and SA).

Objectives:

- Standardization of Bio-priming methods in Leguminous Crops
- Comparative Analysis of Growth Parameters of Non-coated and Coated Leguminous Crops
- Comparative Analysis of Oxidative Stress Level in Non-coated and Coated Leguminous Crops
- Comparative Study of Nitrogen Fixation Capability in non-coated and coated Leguminous crops

METHODOLOGY:

Collecction of seeds of plants:

The seeds of varieties *Cicer arietinum(Chick pea), Cyamopsis tetragonoloba (Guar),* and *Vigna unguiculata (Cowpea)* were collected from RARI and Krishi Vigyan Kendra, Dungarpur, Jaipur (Rajasthan). The seeds were 6 months old and had been stored at 5°C.

Selection and maintainance of Microorganism for Biopriming:

Bacterial culture of *Bradyrhizobium sp.* and *Mesorhizobium ciceri* should be procure from MTCc, Chandigarh and maintained at the optimal temp. and conditions.

Standaridization of Biopriming Methods:

For the standaridization of given varieties *Cicer arietinum(Chick pea), Cyamopsis tetragonoloba (Guar),* and *Vigna unguiculata (Cowpea),* following methods can be used:

1) ***Dry Powder Coating :***

   A seed coating technique called dry powder application is used to coat seeds with dry powder. "Planter box" treatment is the more traditional name for this application method [Ashraf, M,2007]. For bacterial or fungal treatments followed by drying (hydration/dehydration), dry powders, also known as dust, are utilized [Horchani, F.; Hajri, R.; Khayati, Phytol, 2010], and seeds may have a lower shelf life after application. This method can be used for on-farm pest control applications of branded treatments [E.; Hafsi, C.; Guidi,2009]. Equipment and technology for applying dry powder have advanced to enable more precise loading of material onto seedlings. A spinning stainless-steel brush sifts a powder material through a metering screen. [Mhadhbi, H.; Jebara, M.; Limam, F.; Huguet,2005]. The machinery is weight-based and calibrated to distribute powder to a specific weight of seed. Although not depicted in the figure, most delivery methods (such as an auger, conveyor, seed tender, etc.) would move the seed underneath the dry powder applicator .com [Vadez, V.; Rodier, 1996]. This apparatus is utilized for the standalone application of dry powders as well as for the finishing powder applications following seed dressing or film coating. To provide coating powders, finishing powders, or dry powder actives to seed on a volumetric or weight basis, another dry powder feeder device employs a computer-controlled auger with a hopper vibrator [Hardy, R.W.F.; Bums,1973]. Dry powder carriers, which lessen seed-to-seed friction in the planter, may function as lubricants to improve seed flow ability. Talc and graphite are the two most widely used dry powders, however, a new study has shown that soy-based protein is an

economical and environmentally friendly seed lubricant that enhances flow and singulation during planting without generating dust. Thus, using protein derived from soy has the potential to lessen the danger of adverse effects on humans and pollinators. Due to its adhesion to seeds, dry coating powder dosages are constrained and vary from 0.06 to 1.0% of seed weight. Due to the larger seed surface area of smaller seeds, this loading rate is inversely proportional to seed size, and the amount of powder kept rises as seed size decreases.

2) ***Seed Dressing:***

The most popular technique for applying small doses of active ingredients to seeds is seed dressing [Cakmak, I.; Horst, W.J, 1991]. Although there are many different types of coating equipment, the rotary coater is the one that is most frequently utilized. Seeds spinning inside a metal cylinder are covered with liquid and atomized before being ejected after being freshly treated. This technique can be used to apply a wide variety of active ingredients, particularly chemical plant protectants. Typically, liquid seed treatment formulations are dosed between 0.05 and 1.0% by weight. Finishing powders or fluency powders are used right away following the liquid application of greater loading rates of chemical seed treatment, particularly insecticides, to absorb excess liquid [Mhadhbi, H.; Fotopoulos,2009]. The dry finishing powders can be applied directly downstream using the dry seed coating apparatus or placed into the rotary coater while it is running.

3) ***Film Coating***:

A seed coating technique called film coating was developed originally for the pharmaceutical and confectionary industries. The process of film coating entails creating a consistent, thin layer on top of the seed surface. The main seed coating tool for film coating is the rotary coater. Prior to application on seeds, film-coating polymers (liquid components) are designed to dissolve or dispense the active ingredient. Film coating achieved a 90% application recovery rate, with only minor shape and size changes during the process[Isayenkov, S.V.; Maathuis,2009]. The most versatile of all seed application processes, film coating has seen a considerable increase in utilization. The effectiveness of film-coated seed is assessed based on germination and dust suppression. The flow ability of the seed is improved during handling, processing, and sowing activities by film coating. This value-added treatment is favored over traditional approaches because it delivers protectants to valuable seeds very well and has a pleasing aesthetic. Film-coated seed weight increases vary from 2 to 5% of seed weight. A vented pan and fluidized bed seed coating facilitate contemporaneous treatment and drying [T.j. Bot. 2004,], whereas seed weight build-up of more than 5% necessitates other seed coating equipment with drying capabilities while coating. However, compared to the rotary pan technique, both side-ventilated or perforated pans and fluidized beds are utilized far less frequently. Dry finishing powders can be applied to the rotary coater or used with dry seed coating equipment to boost loading from 5-8% as explained for seed treatment. The success of field sowing [Hachicha, M.; Job, J.O.; Mtimet,1993] and environmental protection depend on the selection of film-forming polymers. In comparison to graphite-treated or

uncoated corn seeds, Poly Seed CF (Rigrantec, Porto Alegre, RS, Brazil) coated corn seeds increased precision seed positioning with a notable reduction in dust production and leaching of applied pesticides [James, E.K.,1999]. Additionally, the seed treatment adherence of the film coating polymer was good, which reduced dust off-gassing into the environment. 5% of the weight of the seed [Striker, G.G.; Colmer,2017]. A vented pan and fluidized bed seed coating facilitate contemporaneous treatment and drying, whereas seed weight build-up of more than 5% necessitates other seed coating equipment with drying capabilities while coating. However, compared to the rotary pan technique, both side-ventilated or perforated pans and fluidized beds are utilized far less frequently. Dry finishing powders can be applied to the rotary coater or used with dry seed coating equipment to boost loading from 5-8% as explained for seed treatment. The success of field sowing and environmental protection depends on the selection of film-forming polymers. In comparison to graphite-treated or uncoated corn seeds, PolySeed CF (Rigrantec, Porto Alegre, RS, Brazil) coated corn seeds increased precision seed positioning with a notable reduction in dust production and leaching of applied pesticides. Additionally, the seed treatment adherence of the film coating polymer was good, which reduced dust off-gassing into the environment [James, E.K.; Sprent, J.I.,1999].

4) Encrusting:

Encrusting is a seed-coating technique that involves the addition of liquids and solid particles and produces coated seeds that are thoroughly covered while retaining their original shape. Mini-pellets or coated seeds are other names for encrusted seeds. The rotary coater or coating pan are the two main coating techniques for producing encrusted seed (Figure 1). Because there is a lot of water added during the encrusting process, the freshly coated seed must be dried to its natural seed moisture content before being packaged and stored. After encrusting, the weight might increase by 8 to 500%. It has been demonstrated that encrusted seeds promote seedling emergence. Fescue seed germination was shown to be much higher when seeds were encrusted prior to storage as opposed to after storage or untreated seeds [Aschi-Smiti, S.; Chaibi, W.; Brouquisse, 2003]. Encrusted seed takes longer to germinate than seed that is film-coated, depending on the seed coating thickness or percentage build-up. The amount of binder used to create encrusted coatings alters their mechanical characteristics, such as integrity, compressive strength, and how long they take to break down after soaking. Vs seed with a film coating. The amount of binder used to create encrusted coatings alters their mechanical characteristics, such as integrity, compressive strength, and how long they take to break down after soaking.

5) Pelleting and Agglomeration:

Seed pelleting is a continuation of the encrusting coating process resulting in even greater build-up so that the coated crop seed's natural size or form is hidden [Chaves, M.M.; Flexas, J.; Pinheiro,2009]. However, typical mineral materials described in the literature and patents are offered. The materials and methods employed for this purpose are private. The binders could be dry powders or liquids. To create a coating blend, dry powder

binders and filler components are combined [Zahran, H.H.,1999], with the only liquid needed being water applied during the coating process. After pelleting and drying, the weight gain ranges from 500 to >5000%. A 500% weight increase is a 1:5 build-up of seed to coating since it is typical to describe the percent weight increase as a ratio of seed weight to dry pellet weight.

To ensure that the pelleted seed will germinate without being hampered by the pellet matrix, the choice of liquids and fillers is crucial [Striker and Colmer,2017]. Commercial pelleting products for growers have been the subject of much study and development by the seed pelleting business. Growers are increasingly requesting pelleted seeds so that seeds may be placed precisely. Pelletized seed allows for precise seed spacing, which minimizes the requirement for thinning operations. Plant transplants are frequently grown from pelleted seeds. High-value, small-seeded horticultural crops are regularly pelleted.

Particle size distribution, porosity, water absorption and holding ability, and absence of toxicity are all necessary material qualities for successful pelleting. A mixture of bentonite and talc or pumice was strongly advised for pelleting tobacco seeds. Similar results were obtained for broccoli [Hernandez and Jimenez,2001] and lettuce using diatomaceous earth and a mixture of gypsum and calcium carbonate, respectively. When seeds are sown in soil that is water-saturated and has little oxygen available, calcium peroxide that was supplied as a component of the seed covering [Armstrong,1979] provides oxygen gas to the sprouting seed. Rice under submerged settings benefited from calcium peroxide applied in a seed pellet for better emergence and crop establishment.

Pelleting requires the most time and expertise compared to other coating technologies due to the extensive application of active components, liquids, and solid particulates. The pellet should not cause any restriction to germination when sown in the field. pellet integrity is dependent on the selection of material (fillers and binders) and appropriate technology [Isayenkov and Maathuis,2009].

Morphological and Physiological parameters analysis:

1) Growth Analysis:

The length of the lateral root (LR), the length of the shoot (LS), and the number of nodules(NN) were all variables associated to plant growth . Lateral shoot and Lateral Root will be calculated by measuring the distance between the crown and the leaf tip [cm] and the crown and the root tip [cm], respectively [Benmoussa and Nouairi, 2022].

2) Plant Biomass:

The fresh mass of roots and shoots will be measured on fresh material; the dry mass of roots (DMR) and shoots (DMS) will be determined after desiccation of the samples for three days in a 65◦C oven [Benmoussa and Nouairi,2022].

3) Chlorophyll Content and Gas Exchange Measurement:

Chlorophyll measurement would be achieved as described by Wintermans and Mots. About 100 mg of fresh leaves will be homogenized with a mortar and pestle in 10 mL 80% (v/v) acetone (8 mL acetone with 2 mL distilled water). The mixture will then be strongly shaken and kept at 4◦C for 48h. After centrifugation for 1 min at 20,000×g, the absorbance of the supernatant will be measured at 663 nm for chlorophyll a (Chl a), at 645 nm for chlorophyll b (Chl b) and 470 nm for carotenoids. Total chlorophyll content would come.

4) Photosynthetic Parameters:

As net assimilation rate of CO_2 (A), total transpiration (E), stomatal conductance (Gs), and intercellular CO_2 concentrations (Ci) will be measured using the ADC LCpro+ portable Photosynthesis measuring system (ADC Bioscientific Ltd., Hoddesdon, UK) equipped with different gas chambers, according to manufacturer instructions. The measurements would be done under the following conditions: saturating light intensity of about 1.350 µmol (photon) m−2 s−1, the CO2 concentration of 380 µmol mol−1, leaf temperature of 25±2 ◦C, and relative humidity was 65±5%. The measurement time will be between 11:00 AM and 1:00 PM corresponding to the optimum of the photosynthetic activity [Benmoussa and Nouairi, 2022].

5) The Electrolyte Leakage:

100 mg of leaf discs from the middle part of the freshly cut leaves (three plants per genotype and treatment) were put in assay tubes repleted with 10 mL of ultrapure water. The tubes were incubated in a water bath for two hours at 32 ◦C. Then, the first electrical conductivity (EC1) of the solution was measured using a 712 conductometer (Metrohm). The tubes were then autoclaved at 100 ◦C for 20 min and cooled at ambient temperature, and the second electrical conductivity (EC2) was measured. The electrolyte leakage was measured by the formula EL = (EC1/EC2)×100 [Fan, L.; Zheng and Wang, 1997].

6) Evaluation of Soil Parameters:

Commercial laboratories that offer a range of tests, focusing on particular groupings of chemicals and minerals, frequently carry out soil testing.

Laboratory tests often check for plant nutrients in three categories:

- Major nutrients: Nitrogen (N), Phosphorus (P), and Potassium (K)
- Secondary nutrients: Sulfur
- Minor nutrients: Iron, Copper, and Zinc.
- Physical parameters: pH, EC, and OC

Oxidative Stress level Analysis :

1) ***Lipid Peroxidation, H_2O_2, Chlorophyll, and Non-enzymatic antioxidant Proline determination:***

The degree of lipid peroxidation will be determined by the method of De Vos et al, (1989). Lipid peroxidation was evacuated by examination of the malonaldehyde (MDA) content using an extinction coefficient of 155 mM^{-1} CM^{-1}.
H2O2 content will be samples from both plants using methods of Alexieva et al, (2001) are measured. The amount of hydrogen peroxide is calculated using a standard curve generated at known concentrations of H2O2.

To estimate chlorophyll in fresh leaves *of* seedlings will be collected for the determination of pigments according to the processed Arnon (1949).

The proline content will be calculated in two plants by the method of Bates et al., (1973) it was determined through the standard proline curve.

Estimation Biochemical assay for checking the activity of antioxidant enzymes:

- **Extraction of antioxidant enzymes:**
- For the preparation of extracts for the determination of SOD, CAT, and APX, GR activities, the tissue will be homogenized under ice-cold conditions in 3ml extraction buffer. This mixture will be centrifuged; the resulting supernatant is stored at -20 ° C for testing various antioxidant enzymes.
- **Enzyme assays and protein determination:**

- The activity will be SOD is assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium by the method of Beauchamp and Fridovich,(1971).CAT activity will be measured by the method of Aebi, (1974).

- The APX activity is the ascorbic acid-dependent H_2O_2 oxidation rate. Its activity will be determined in a reaction mixture, and the extract of enzyme phosphate buffer and ascorbic acid would contain (Chen and Asada, 1989).

- GR will be prepared by the method of Smith et al., (1988). This method is based on increasing the absorbance at 412 nm, when 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced by GSH.

- The POD will be test is performed according to the Putter method (1974). By regulating the production of Tetraguaiacol at 436 nm during the enzymatic reaction of the starting substrate, guaiacol, in the presence of H2O2, Activity POD will be ascertained.

- The protein content of *Cyamopsis tetragonoloba* (L.) Taub and *Vigna radiata* (L.) will be seedlings by the method of Lowry et al., (1951).

2) Lipid Peroxidation Assay:

Lipid peroxidation will be estimated according to Cakmak and Horst [33]. 0.5 mg of fresh leaf sample (three plants per genotype and treatment) were homogenized in 0.1% (w/v) trichloro-acetic acid (TCA) solution and centrifuged at 12,000×g for 15 min at 4◦C. The supernatants would be recovered, supplemented with 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) (TCA), and incubated at 100 ◦C for 20 min. Then the samples will be cooled in an ice bath and centrifuged at 10,000×g for 5 min. In addition, the absorbance of the supernatants would read at 532 nm, the value for non-specific absorption at 600 nm will be subtracted to the 532 nm value, and the concentration of malondialdehyde-TBA complex (MDA-TBA) would be calculated using an extinction coefficient of 155 Mm^{-1} cm^{-1}.

Nitrogen Fixation Analysis:

1) Nodulation Assay:

Seeds of the selected crops will be surface sterilized with 70% alcohol and 2% sodium hypochlorite, then thoroughly washed five times with sterile distilled water. After imbibitions in sterile distilled water, seeds would be retained in darkness at 4◦ Cover night. Then, seeds will germinate in autoclaved perlite moistened with sterile tilled water for 3 days at 28◦C according to Mhadhbi et al. [2005]. After germination, seeds would be sowed into plastic pots (500 mL) filled with autoclaved sand. Plants should be inoculated with one rhizobia strains (out of 20 rhizobia strains), and the control plants would be nitrogen fertilized with KNO3 as a source of nitrogen without any bacterial inoculation. Nine plants in nine different pots will be used for every strain. Two inoculations were performed after the transfer of the seeds to the pots. The first inoculation would be carried out two weeks after the transfer and the second one after one month. Plants were grown for sixty days in a growth chamber under controlled conditions (16/8 h light/darkness, 19 ◦C in the dark with a relative humidity (RH) of 60% and 25◦C in the light with an RH of 80%, photosynthetic photon flux density of 300 µmol m−2 s−1) and watered with a nitrogen-free nutrient solution as described by Vadez et al. [1996]. Root and shoot length, nodule number, and ARA will be independently measured on the six replicates.

2) Acetylene Reduction Assay (ARA):

Nitrogen fixation capacity will be measured by acetylene reduction assay (ARA), in situ, using gas chromatography with a Porapak-column, according to Hardy et al. [1973]. As detailed in Mhadhbi et al. [2009], pure acetylene and ethylene would be used as external standards.

Experimental Soils:

Three composite soils were collected from North-Easteren Rajasthan. The three local areas are;

I. Vill. Jharna, Jaipur
II. Vill. Mahlan, Jaipur
III. Bhiwadi, Alwar

The main Physicochemical characteristics of the three soils that will be reported are Conductivity, Organic material, Total organic carbon(TOC), Total nitrogen, and Total phosphorus.The organic matter content, total phosphorus, total nitrogen, total organic carbon

(TOC), potassium, and conductivity in the soils will be determined according to the protocols described in SSICA.

RESEARCH QUESTIONS

1. What is the role of seed priming to enhance growth and development of crop plants against biotic and abiotic stresses?
2. Does seed priming is a good approach towards agricultural sustainability?

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